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Assessing the aerodynamic diameters of taxon-specific fungal bioaerosols by quantitative PCR and next-generation DNA sequencing

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Abstract

Aerodynamic diameter is an important determinant of the physical processes that act upon airborne fungi. Processes include gravitational settling, respiratory deposition, penetration into buildings, resuspension from surfaces into air, and long-range transport. This study combined next-generation DNA sequencing (NGS) with quantitative PCR (qPCR) to evaluate diverse, taxon-specific, fungal aerodynamic diameters from bioaerosol samples. The accuracy of the method was demonstrated by comparing geometric mean aerodynamic diameters of selected taxa produced by the NGS-based method to the diameters produced by taxon-specific qPCR (r = 0.996). Geometric means (d_g) and geometric standard deviations (σ_g) of aerodynamic diameters were characterized for more than 50 fungal taxa, spanning 55 genera, 9 classes, and 2 phyla. The results reported in this study demonstrate the robust nature of this method, provide novel insights into aerodynamic properties of diverse airborne fungal species, and potentially enable a better accounting of taxon-specific fungal fate and exposure both in indoor air and in the atmosphere.

Keywords: Fungi, Internal transcribed spacer (ITS), Pyrosequencing, Aerodynamic diameter, Quantitative PCR (qPCR), Bioaerosols

1. Introduction

Aerodynamic diameter exerts significant influence over the important physical processes that act upon airborne biological particles. These processes include but are not limited to ice nucleation, gravitational settling, respiratory deposition, penetration into buildings, resuspension into air, and long-range transport (Ariya *et al.*, 2009; Nazaroff, 2004; Prospero *et al.*, 2005; Riley *et al.*, 2002; Thatcher & Layton, 1995; Yeh *et al.*, 1996). A particle's aerodynamic diameter is the diameter of a sphere with a density of 1 g/cm^3 that has the same settling velocity as the particle of interest. Traditionally, the aerodynamic diameters of airborne fungi have been characterized by means of enumerating culturable organisms that were captured on multistage cascade impactors (Madelin & Johnson, 1992; McCartney *et al.*, 1993; Reponen, 1995) or by time-of-flight (TOF)-based aerodynamic particle sizing (Han *et al.*, 2011; Madelin & Johnson, 1992; Reponen *et al.*, 1996). These methods have important limitations for characterizing fungal aerodynamic diameter in indoor air or in the outdoor atmosphere. Impactor data derived from culturing is restricted to species that can be readily identified, and cannot account for nonviable fungal spores and fragments, or fungi that are not culturable under the given conditions (Peccia & Hernandez, 2006; Reponen, 1995). TOF-based methods do not allow for the identification of fungal taxa or the discrimination of fungal particles from nonfungal particles. Thus, their use is limited to fungal aerosol studies that start from pure cultures.

Fungi are remarkably diverse with an estimated 1.5 million species (Bass & Richards, 2011; Blackwell, 2011; Hawksworth, 2001). Recent airborne fungal diversity analyses based on fungal barcoding via next-generation DNA sequencing (NGS) has resulted in the identification of hundreds to thousands of different fungal taxa in outdoor

aerosol samples (Adams *et al.*, 2013; Dannemiller *et al.*, 2014; Yamamoto *et al.*, 2012). When coupled with size-resolved sampling, the NGS/fungal barcoding technique may enable approaches to determine the aerodynamic diameters of bioaerosols associated with specific fungal taxa. However, NGS-based data provides taxon results as a relative abundance per size bin. Such data must be transformed into absolute concentrations to assess the distribution of aerodynamic diameters for the different phyla, genera, or species that are identified.

The goal of this study was to demonstrate an approach using NGS/fungal barcoding and universal qPCR data to characterize taxon-specific fungal aerodynamic diameters in environmental aerosol samples. Relative abundance results were coupled with universal qPCR data from particle-size-fractionated outdoor air samples collected at a site in the northeastern United States. Absolute concentrations of each fungal taxon in each size fraction were obtained by multiplying universal fungal qPCR results by the NGS-based relative abundance data (Dannemiller *et al.*, 2014). Geometric mean aerodynamic diameters and geometric standard deviations for bioaerosols associated with specific taxa were computed. These values were then compared to geometric mean aerodynamic diameters determined by taxon-specific qPCR. This study provides, for the first time, a robust approach for quantifying geometric means and geometric standard deviations of taxon-specific fungal aerodynamic diameters for aerosol particles based on NGS and qPCR data, broadening the potential to determine the aerodynamic diameters for fungal taxa in air.

2. Experimental

2.1. Seasonal fungal study

The study utilized data and samples from a seasonal sampling campaign conducted in New Haven, Connecticut, USA (41°18'29"N 72°55'43"W) in 2009-2011 (Yamamoto *et al.*, 2012). Airborne fungi were collected at a continuous sampling rate of 28.3 l min^{-1} for ~ 4-week sampling periods in each of four seasons. We sampled onto glass fiber filter substrates using an eight-stage non-viable Andersen sampler with aerodynamic diameter (d_a) cutoffs of 0.4, 0.7, 1.1, 2.1, 3.3, 4.7, 5.8, and 9.0 μm. Glass fiber substrates were used to minimize particle bounce (Hu, 1971). The data used here from the previous study included the taxonomic libraries for the different impactor stages for the four different seasons and taxon-specific qPCR data for *Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, and *Penicillium chrysogenum.* To estimate the absolute concentration for specific taxa, total fungal qPCR was carried out in the present study using DNA extracts of the previously collected seasonal samples. Below, brief descriptions of the methods performed in the prior study are provided along with a more in-depth description of the universal qPCR methods and estimation of aerodynamic diameters for specific taxa.

2.2. Universal fungal qPCR

Total fungal concentrations were measured by universal fungal qPCR with primers FF2 (5'-GGTTCTATTTTGTTGGTTTCTA-3') and FR1 (5'-CTCTCAATCTGTCAATCCTTATT-3') (Zhou *et al.*, 2000). Reaction mixtures totaling 50 μL included template DNA (2 μL), 1× SYBR Green Master Mix (FastStart Universal SYBR Green Master (ROX); Roche Applied Science) and 0.3 μM of each

primer. A real-time PCR system (ABI 7500 Fast Real-time PCR System; Applied Biosystems) was used with these thermal conditions: 50 ˚C for 2 min, 95 ˚C for 15 min of initial denaturation and 45 cycles of 95 ˚C for 15 s of dissociation and 60 ˚C for 1 min of annealing and extension. Threshold cycles were determined by the ABI 7500 auto function. The results were calibrated against an *Aspergillus fumigatus* standard. Thus, reported universal fungal qPCR results are based on *A. fumigatus* spore equivalents (SE) per m^3 as previously described (Dannemiller *et al.*, 2014; Hospodsky *et al.*, 2010; Lang-Yona *et al.*, 2012).

2.3. Species-specific fungal qPCR

Fungal aerodynamic diameters assessed by the NGS-based method were compared with those characterized by taxon-specific qPCR. Fungal genera of *Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum*, and *Penicillium* were selected for this comparison. *Aspergillus* and *Penicillium* produce small unicellular amerospores, whereas *Alternaria* and *Epicoccum* produce large multicellular dictyospores. *Cladosporium* spp. produces both unicellular and multicellular spores. These taxa were selected to cover a wide range of fungal spore size. Concentrations of *Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, and *Penicillium chrysogenum* were quantified by the reported species-specific qPCR assays (Haugland *et al.*, 2004; Meklin *et al.*, 2004), and their geometric means (d_g) and geometric standard deviations of aerodynamic diameters, calculated according to the method described below, were 10.6 μm (1.55), 3.88 μm (1.25), 4.62 μm (1.46), 11.0 μm (1.61) and 3.89 μm (1.66), respectively. These species-specific d_g values were compared with the corresponding genus-specific aerodynamic diameters as determined by NGS.

The genus-specific values were used for NGS since the numbers of sequences were small for *A. alternata*, *A. fumigatus*, and *P. chrysogenum* (< 15 sequences) if the results were analyzed at the species level.

2.4. Next-generation DNA sequencing and taxonomic assignment

The well-established methods used for the NGS/barcoding methodology are described elsewhere (Yamamoto *et al.*, 2012). Briefly, the internal transcribed spacer (ITS) region of fungal DNA is targeted as a barcode marker for identification (Schoch *et al.*, 2012). The extracted DNA was amplified for the ITS sequences circumscribed by universal fungal primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Larena *et al.*, 1999; Manter & Vivanco, 2007). The purified amplicons were normalized and sequenced on the 454 GS FLX Titanium Platform (454 Life Sciences) at the Yale University Center for Genome Analysis. The samples with d_a < 2.1 μm were not included for the sequencing analyses owing to no or weak PCR amplification. Overall, 15,326 ITS sequences were produced. Two phyla, 19 classes, and 558 genera were identified, and the relative abundances were calculated at the phylum, class, and genus ranks (Yamamoto *et al.*, 2012). Taxa with at least 40 ITS sequences detected were included for subsequent analyses of particle size distributions.

2.5. Evaluating aerodynamic diameters

A schematic diagram of a method to assess fungal aerodynamic diameters from the bioaerosol samples is shown in Fig. 1. NGS provides relative abundance fractions of each taxon within a sample (Fig. 1A), whereas the universal fungal qPCR measures absolute concentrations of total fungi (Fig. 1B). Thus, absolute

concentrations of each taxon (N_{taxon}) are determined by the following equation:

$$N_{taxon} = F_{taxon} \times N_{total} \quad (1)$$

where F_{taxon} is the relative abundance fraction of a taxon obtained by NGS and N_{total} is the absolute concentration (SE m^{-3}) of total fungi measured by the universal fungal qPCR. The absolute concentrations of each taxon were calculated for each particle size range (Fig. 1C), and geometric means (d_g) and geometric standard deviation (σ_g) of aerodynamic diameters were calculated for each taxon (Fig. 1D).

As reported in the results section, the taxon-specific d_g values ranged widely from < 2.1 μm to 11.8 μm. For some taxa, particle size distributions were left- or right-truncated, which potentially results in inaccurate d_g and σ_g estimations if traditional forward-calculation methods are used (Yamamoto *et al.*, 2012). Here we used a best-fit d_g and σ_g method which has the advantage of not requiring knowledge of the upper size limit on the largest bin, and does not utilize any *a priori* assumptions about the distribution within each size bin. In this method, we executed a search procedure to solve for the best-fit values of d_g and σ_g under the assumption that the sampled size distributions of taxon-specific fungal DNA were lognormal. The procedure aimed to minimize the residual between predicted and measured particle size distributions (Fig. 2) by using the least-squares method. To avoid the risk of settling on a local minimum, all possible combinations of d_g (0.4 to 15 μm in steps of 0.01 μm) and σ_g (1.01 to 3 in steps of 0.01) were tested. The computations were executed using Excel Visual Basic for Applications ver. 7.0, which can be downloaded at https://sourceforge.net/projects/gmcalculator/.

For most taxa, we used the seasonally averaged particle size distributions as the input for computing values of d_g and σ_g. For some taxa, the largest relative

abundances were in the winter; however, the absolute fungal DNA concentrations were the lowest in the winter. In some of these cases, we found a high residual when computing the geometric parameters for annual averaged data. Thus, in cases in which the residual between the predicted and measured particle size distributions was greater than 20% for the annual average data and the greatest relative abundance was observed in the winter, only the winter data were used to compute d_g and σ_g. The distributional parameters for these taxa were excluded from the final reporting if the residuals also were greater than 20% using the winter data. Some taxa showed a residual greater than 20%, and the greatest relative abundance was not found in the winter. However, none of these taxa was found to show a residual smaller than 20% even when selecting only the most abundant season.

2.6. Count median diameter (CMD) estimation

The DNA-based methods provide particle size distributions of airborne fungi based on the third moment of particle size distribution (i.e., corresponding to mass distributions). To allow for comparisons with culture-based literature data that reflect the first moment of particle size distributions (i.e., corresponding to count distributions), we also estimated count median diameters (CMD) for geometric means of fungal aerodynamic diameters characterized by the NGS-based method and for species-specific qPCR. To estimate CMD, the Hatch-Choate equation was used (Hinds, 1999):

$$\mathrm{CMD} = d_g \exp\left(-3\ln^2 \sigma_g\right) \quad (2)$$

3. Results

3.1. Comparing the NGS-based method and species-specific qPCR

Fig. 3 shows the relationship of fungal aerodynamic diameters characterized by the NGS-based method and by species-specific qPCR. Only the winter data were used for *Aspergillus* and *Penicillium* since large residuals between the measured and predicted particle size distributions (>20%) were observed for the annual averages. The d_g values by the species-specific qPCR were 10.6, 3.88, 4.62, 11.0 and 3.89 μm for *Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum*, and *Penicillium*, respectively. The NGS-based method produced the respective d_g values of 10.6, 5.16, 5.52, 11.8, and 5.07 μm. Although different taxonomic ranks were used for the comparison, a strong correlation was observed between the two methods (Pearson's $r = 0.996$, $p < 0.001$). The σ_g values were also consistent between these two methods (Table 1), substantiating the capability of the NGS-based method to determine, with reasonable accuracy, taxon-specific fungal aerodynamic diameters.

3.2. Comparing aerodynamic diameters of different methods

Table 1 shows the values of d_g and σ_g of selected fungal genera obtained by different methods. The d_g values measured by NGS and species-specific qPCR were consistently greater than those reported from culture-based methods. To estimate the d_g values based on the moment of count distributions, count median diameters (CMD) were estimated for aerodynamic diameters obtained by the NGS- and species-specific qPCR-based methods by Eq. (2). The resulting CMD values were correlated with, but smaller than the original d_g values (Pearson's $r = 0.772$, $p < 0.001$) and more similar to the d_g values characterized by the growth-based method (Table 1).

3.3. Taxon-specific particle size distributions

Two phyla, 19 classes, and 558 genera were identified (Yamamoto *et al.*, 2012), of which 2 phyla, 9 classes, and 55 genera were found to have (a) more than 40 ITS sequences, and (b) residual errors between predicted and measured size distributions smaller than 20%. At the phylum rank, geometric means and standard deviations of aerodynamic diameters were 7.68 μm (1.73) for *Ascomycota* and 4.44 μm (1.43) for *Basidiomycota* (Fig. 1D) when data for all seasons were pooled. The season-specific values for *Ascomycota* were 8.28 μm (1.86), 5.75 μm (1.73), 8.59 μm (1.45), and 7.55 μm (1.69) in the spring, summer, fall, and winter, respectively, whereas the respective values for *Basidiomycota* were 4.85 μm (1.38), 3.61 μm (1.37), 5.06 μm (1.41), and 4.42 μm (1.24).

Fig. 4 illustrates particle size-resolved taxonomic compositions of airborne fungi. The class *Agaricomycetes* of the phylum *Basidiomycota* represented the largest fraction (45%), whereas the class *Dothideomycetes* of the phylum *Ascomycota* accounted for the second largest fraction (35%). The dominance of these two classes was consistent throughout each season. Their geometric mean diameters varied seasonally with the smallest values observed in the summer. The season-specific d_g and σ_g values for *Agaricomycetes* were 4.78 μm (1.34), 3.59 μm (1.37), 4.88 μm (1.37), and 4.38 μm (1.23) in the spring, summer, fall, and winter, respectively, whereas the respective values for *Dothideomycetes* were 8.03 μm (2.06), 6.08 μm (1.78), 9.22 μm (1.43), and 10.4 μm (1.84).

Tables 2 and 3 list d_g, σ_g, and the estimated CMD of aerodynamic diameters of the 9 most abundant classes and 55 most abundant genera, respectively. The d_g values

varied substantially across the fungal taxa, ranging from < 2.1 μm for *Antrodia* to 11.8 μm for *Epicoccum*. Large d_g values were observed for the *Ascomycota* genera of *Alternaria* (10.6 μm), *Epicoccum* (11.8 μm), *Leptosphaerulina* (9.55 μm), and *Monilinia* (9.68 μm). The d_g values smaller than 3.3 μm were observed for the *Basidiomycota* genera of *Antrodia* (< 2.1 μm), *Phlebia* (3.04 μm) *Sistotrema* (2.26 μm) and *Wallemia* (3.01 μm).

4. Discussion

Although the aerodynamic diameter is an important microbial feature that influences fungal aerosol source emissions, deposition rates and environmental fate, and human exposure, this parameter has not been well characterized in prior studies for relevant fungal taxa in an environmental setting. The present study demonstrates an approach for determining the aerodynamic diameters of a broad diversity of fungal taxa (2 phyla, 9 classes, and 55 genera) suspended in bioaerosols. The method, applied here to atmospheric samples, is also appropriate for indoor air studies.

The reported NGS-based method produced fungal particle-size distributions that were highly consistent with those characterized by taxon-specific qPCR (Fig. 3). Given that taxon-specific qPCR has established accuracy as a reference method (Haugland *et al.*, 2004; Meklin *et al.*, 2004), the results indicate the capability of the NGS-based method to accurately assess fungal aerodynamic diameters.

Application of this approach using size-resolved relative abundance data from prior sampling campaigns revealed a diversity of aerodynamic diameters among taxa. Taxon-dependent d_g values are expected owing to fungal physiology, physical spore sizes, method of spore release, and environmental fate and transport that are unique to

each fungal group. At the phylum rank, average geometric means of aerodynamic diameters were 7.68 μm for *Ascomycota* and 4.44 μm for *Basidiomycota* (Fig. 1). The dominant classes of the *Ascomycota* and *Basidiomycota* phyla were *Dothideomycetes* and *Agaricomycetes*, respectively (Fig. 4). Large proportions of *Dothideomycetes* and *Agaricomycetes* in outdoor air were also reported in a previous sequencing-based study (Fröhlich-Nowoisky *et al.*, 2009). The four most abundant genera of the class *Dothideomycetes* and their d_g values were *Leptosphaerulina* (9.55 μm), *Epicoccum* (11.8 μm), *Cladosporium* (5.52 μm), and *Alternaria* (10.6 μm) (Table 3). These fungi produce large multicellular dictyospores with reported spore sizes of 24–36×10–14, 15–25, 3–11×2–5, 18–83×7–18 μm, respectively (Cole & Samson, 1984; Mitkowski & Browning, 2004). Meanwhile, the four most abundant genera of the class *Agaricomycetes* and their d_g values were *Peniophora* (4.41 μm), *Exidia* (5.70 μm), *Stereum* (4.13 μm), and *Trametes* (3.34 μm) (Table 2). Their reported spore sizes are 6.5–8×3–3.5, 2–4×1, 2.5×6–7, and 6.6–9.2×2.4–3 μm, respectively (Burt, 1920; Ingold, 1995; Li & Cui, 2010; Whelden, 1936).

As described in the results section, seasonal variations in the aerodynamic diameters have been observed, with the smallest values found in the summer. The observed tendency was consistent with results using species-specific qPCR in our previous study (Yamamoto et al., 2012). Though the mechanisms are unknown, the smaller spores might be produced during summer owing to higher temperature (Phillips, 1982). The finding may be clinically relevant as changes in spore sizes can affect inhalability and respirability of allergenic and pathogenic airborne fungal spores (Reponen, 1995).

Comparing the aerodynamic diameters of fungal bioaerosols with spore sizes

derived in a prior culture-based environmental study reveals that the geometric mean aerodynamic diameters estimated from the qPCR and NGS methods are greater (Table 1). A possible cause of this finding is the agglomeration of fungal spores in the atmosphere or in indoor air (Heikkila *et al.*, 1988; Lacey, 1991). Using culture-based methods, a single aerosol dispersal unit that contains multiple spores may develop into only one colony and thus result in one identifiable fungal count per dispersal unit. NGS and qPCR methods, in contrast, quantify the multiple spores in the above dispersal unit, thus assigning a value greater than one fungal count to this larger, aggregate particle. Consequently, the moments of particle size distributions are different, and molecular techniques may produce a larger d_g value than culture-based techniques. In addition, comparing the qPCR- and NGS-based d_g values or the culture based d_g values from environmental studies (Table 1) with pure culture TOF-based data (*Aspergillus fumigatus* d_g = 2.15 μm, *Penicillium chrysogenum* d_g = 2.8 μm, and *Cladosporium cladosporioides* d_g = 1.8 μm) (Madelin & Johnson, 1992; Reponen *et al.*, 1996) reinforces a finding that many fungal spores sampled from the atmosphere or indoor air are not in the form of single isolated spores. The difference may also be attributable to different environmental samples. In each environment, the sizes of airborne fungal spores or DNA may vary by attachment to other abiotic particulate matter (Lighthart, 1997; Yamaguchi *et al.*, 2012). Finally, the existence of nonculturable fungal fragments that produce an NGS/qPCR signal might further differentially impact the observed particle size statistics of fungal aerosols (Peccia & Hernandez, 2006).

Limitations in the present method for determining taxon-specific size characteristics center upon assumptions made in converting relative abundance values to absolute concentrations. The accuracy of this taxon-specific concentration

estimation has previously been described (Dannemiller *et al.*, 2014). While the NGS/qPCR methods for determining taxon-specific concentration are strongly correlated with taxon-specific qPCR results from the same sample (Pearson's $r = 0.996$ $p < 0.001$), systematic biases have been observed in the conversions of a quantity of a reference fungal strain into absolute quantities of different fungal taxa (Dannemiller *et al.*, 2014). Using a single strain of *A. fumigatus* for universal qPCR calibration, while necessary, was expected to cause biases owing to taxon-dependent variations in the numbers of rDNA copies per fungal genome (Maleszka & Clarkwalker, 1993; Rooney & Ward, 2005).

Notably, unlike taxon-specific concentration calculation, determination of particle size metrics such as d_g and σ_g by the NGS/qPCR method appear to be less sensitive to a bias associated with copy number variation of fungal ITS. Indeed, a strong correlation between the NGS/qPCR method and taxon-specific qPCR for quantifying the d_g values has been observed (Fig. 2). This outcome is expected due to the nature of the d_g and σ_g calculations, which use the relative proportion of absolute concentrations of each fungal taxon quantified across each particle size bin. Though strain-dependent variation has been reported in the numbers of rDNA copies in *A. fumigatus* (Herrera *et al.*, 2009), our findings indicate that particle size is not a major metric systematically influenced by variations in the numbers of ITS copies. Additional future benefits can be anticipated from ongoing improvements in fungal databases, NGS identification accuracy, and a better understanding of the number of ITS genes in the genomes of a diversity of fungal species (Yamamoto & Bibby, 2014; Yamamoto *et al.*, 2014).

5. Conclusions

Aerodynamic diameter is of central importance for determining physical processes that influence airborne particles, with implications for fungal ecology, human exposure, plant pathogen transport, and climate. Traditionally, aerodynamic diameters of airborne fungi have been studied based on TOF- or growth-based techniques. Taxon-specific qPCR, culturing, or the use of TOF techniques significantly limits the extent of taxon-specific aerodynamic diameters that can be determined in environmental aerosols. The present study used a new approach combining NGS and universal fungal qPCR to evaluate aerodynamic diameters of multiple fungal taxa. The method characterized particle-size distributions of 55 specific fungal genera with a single set of NGS and universal fungal qPCR data, providing important information about the aerodynamic properties of diverse airborne fungal DNA. This new method expands the scope of fungal genera bioaerosol sizes. By avoiding the culture-based underestimation of fungal spore in aggregate, the method also results in larger mean particle sizes than previously reported by culture-based analysis.

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Table 1

Geometric means (d_g, μm) and geometric standard deviations (σ_g) of aerodynamic diameters of airborne fungi obtained by different methods. [a]

	NGS			Species-specific qPCR			Culture		Microscopy
Genera	d_g	σ_g	Estimated CMD	d_g	σ_g	Estimated CMD	d_g	σ_g	L×W (μm)
Aspergillus	5.16	1.20	4.67	3.88	1.25	3.34	1.8 [b]	1.5 [b]	2–3.5 [c]
Penicillium	5.07	1.20	4.59	3.89	1.66	1.80	2.3 [b]	1.2 [b]	2.5 [d]
Cladosporium	5.52	1.58	2.95	4.62	1.46	3.01	2.8 [b]	1.1 [b]	3–11×2–5 [c]
Alternaria	10.6	1.45	7.00	10.6	1.55	5.97	n.a.	n.a.	18–83×7–18 [c]
Epicoccum	11.8	1.54	6.76	11.0	1.61	5.59	n.a.	n.a.	15–25 [c]

[a] Count median diameters (CMD, μm) are estimated for the NGS- and species-specific qPCR-based methods by Eq. (2). The microscopy-based sizes of fungal spores are also listed. For the qPCR and microscopy data, the values specific for the species *Aspergillus fumigatus*, *Penicillium chrysogenum*, *Cladosporium cladosporioides*, *Alternaria alternata*, and *Epicoccum nigrum* are shown. For the NGS and culture data, the genus-specific values are listed. Abbreviations: n.a., not available in the literature.

[b] Reponen (1995).

[c] Cole & Samson (1984).

[d] Madelin & Johnson (1992).

Table 2

Geometric means (d_g) and geometric standard deviation (σ_g) of fungal aerodynamic diameters evaluated by the NGS-based method, showing the seasonally averaged data for the 9 most abundant classes.

Phylum	Class	d_g (μm)	σ_g	Residual [a] (%)	Estimated CMD [b] (μm)
Ascomycota	*Dothideomycetes*	7.94	1.80	4.7	2.82
	Eurotiomycetes	5.38	1.49	13.8	3.34
	Lecanoromycetes	9.71	1.62	2.4	4.83
	Leotiomycetes	7.55	1.51	4.3	4.54
	Sordariomycetes	6.16	1.60	5.1	3.18
Basidiomycota	*Agaricomycetes*	4.35	1.40	4.0	3.10
	Tremellomycetes	6.98	1.69	4.1	3.06
	Microbotryomycetes	6.19	1.55	2.0	3.48
	Wallemiomycetes	3.12	1.42	5.9	2.16

[a] Residual is the square root of the sum of the squared deviations between the predicted and measured concentrations of each size bin of the particle size distribution.

[b] Count median diameters (CMD) are estimated by Eq. (2).

Table 3

Geometric mean (d_g) and geometric standard deviation (σ_g) of fungal aerodynamic diameters evaluated by the NGS-based method, showing the seasonally averaged data for the 55 most abundant genera.

Phylum	Class	Genus	d_g (μm)	σ_g	Residual [a] (%)	Estimated CMD [b] (μm)
Ascomycota	*Dothideomycetes*	*Alternaria*	10.6	1.45	1.1	7.00
		Cladosporium	5.52	1.58	2.2	2.95
		Epicoccum	11.8	1.54	2.2	6.76
		Eudarluca	4.99	1.71	19.0	2.10
		Leptosphaerulina	9.55	1.58	7.2	5.10
		Lophiostoma	6.00	1.44	11.3	4.03
		Mycosphaerella	5.26	1.47	15.5	3.37
		Phaeothecoidea	8.12	1.52	7.3	4.80
		Ramularia	4.89	1.28	10.2	4.07
		Teratosphaeria	9.02	1.67	2.4	4.10
	Eurotiomycetes	*Aspergillus* [c]	5.16	1.20	1.1	4.67
		Eurotium	4.36	1.14	3.4	4.14
		Penicillium [c]	5.07	1.20	4.8	4.59
		Phaeococcomyces	8.26	1.37	4.6	6.14
	Leotiomycetes	*Allantophomopsis*	8.16	1.48	12.6	5.15
		Botryotinia	8.16	1.38	0.8	5.98
		Monilinia	9.68	1.45	3.4	6.40
		Trimmatostroma	7.34	1.56	18.6	4.06
	Sordariomycetes	*Biscogniauxia* [c]	5.02	1.12	0.2	4.83
		Colletotrichum	3.55	1.32	9.0	2.82
		Daldinia	7.68	1.35	7.1	5.86
		Diatrype	7.70	1.48	2.6	4.86
		Eutypa	7.05	1.14	4.0	6.70
		Eutypella	6.23	1.41	3.3	4.37
	Incertae sedis	*Microcyclospora*	9.77	1.49	1.3	6.06
Basidiomycota	*Agaricomycetes*	*Antrodia*	<2.1	n.d.	2.4	n.d.
		Cerrena	4.35	1.21	5.0	3.90
		Coprinellus	5.38	1.21	7.4	4.82
		Cortinarius	5.61	1.22	6.3	4.98
		Cylindrobasidium	5.95	1.33	3.6	4.66
		Daedaleopsis	3.74	1.16	3.4	3.50
		Exidia	5.70	1.19	13.1	5.21
		Ganoderma	5.13	1.15	2.2	4.84
		Hymenochaete	3.66	1.22	3.0	3.25
		Hyphoderma	4.86	1.16	11.3	4.55
		Lycoperdon	4.38	1.16	3.6	4.10
		Mycena	6.75	1.28	6.9	5.62
		Oxyporus	4.00	1.16	6.8	3.74
		Panellus	3.52	1.34	16.9	2.72
		Peniophora	4.41	1.27	7.7	3.72
		Perenniporia	4.17	1.25	7.1	3.59
		Phlebia	3.04	1.46	5.1	1.98
		Piptoporus	2.50	2.07	15.5	0.51
		Pleurotus	5.21	1.33	9.6	4.08

		Polyporus	5.02	1.42	12.3	3.47
		Schizophyllum	3.96	1.39	2.8	2.86
		Sebacina	5.44	1.17	10.2	5.05
		Sistotrema	2.26	2.24	5.2	0.32
		Stereum	4.13	1.32	9.7	3.28
		Trametes	3.34	1.46	3.8	2.17
		Trichaptum	3.85	1.34	3.4	2.98
	Tremellomycetes	*Cryptococcus*	7.71	1.65	4.2	3.63
		Dioszegia	5.55	1.44	10.9	3.72
		Hannaella	9.47	1.75	5.1	3.70
	Wallemiomycetes	*Wallemia*	3.01	1.46	7.0	1.96

[a] Residual is the square root of the sum of the squared deviations between the predicted and measured concentrations of each size bin of the particle size distribution.

[b] Count median diameters (CMD) are estimated by Eq. (2).

[c] The values are based on the winter data alone.

Abbreviation: n.d., not determined owing to the left-truncated particle size distributions.

Figure Legends

Fig. 1. Geometric means (d_g) and geometric standard deviations (σ_g) of taxon-specific fungal aerodynamic diameters as assessed by the next generation DNA sequencing (NGS)-based method. Relative abundances of each fungal taxon were determined by NGS (A), whereas particle size distributions of total airborne fungi were obtained by universal fungal qPCR (B). The NGS-derived relative abundances of each fungal taxon were multiplied by the qPCR-derived particle size distributions of airborne total fungi (C) and the resulting taxon-specific particle size distributions were produced to compute d_g and σ_g for each fungal taxon (D). The values shown are for the phyla *Ascomycota* and *Basidiomycota*, with data pooled for all four seasons.

Fig. 2. Comparison between the measured and predicted particle size distributions. The values of geometric means (d_g) and geometric standard deviation (σ_g) of aerodynamic

diameters were obtained by an analysis that minimized the residual between the predicted and measured particle size distributions. The particle size distributions shown are for *Cladosporium*.

Fig. 3. Comparison of geometric mean of aerodynamic diameters of selected fungal taxa characterized by the species-specific qPCR and the NGS-based method. Each datapoint is an average for four seasons, with the exception of *Aspergillus* and *Penicillium* where only winter data were use due to the large residuals (> 20%) observed for annual averages.

Fig. 4. Particle size-resolved taxonomic compositions of airborne fungi in New Haven, Connecticut, USA in 2009-2011.

Highlights (max 85 characters for each of 3 to 5 bullet points)

- Study combined NGS and qPCR to evaluate the aerodynamic diameters of fungal taxa
- Aerodynamic diameters determined for >50 fungal genera in atmospheric bioaerosols
- Good agreement obtained between diameters estimated by NGS and taxon-specific qPCR

Figure 1

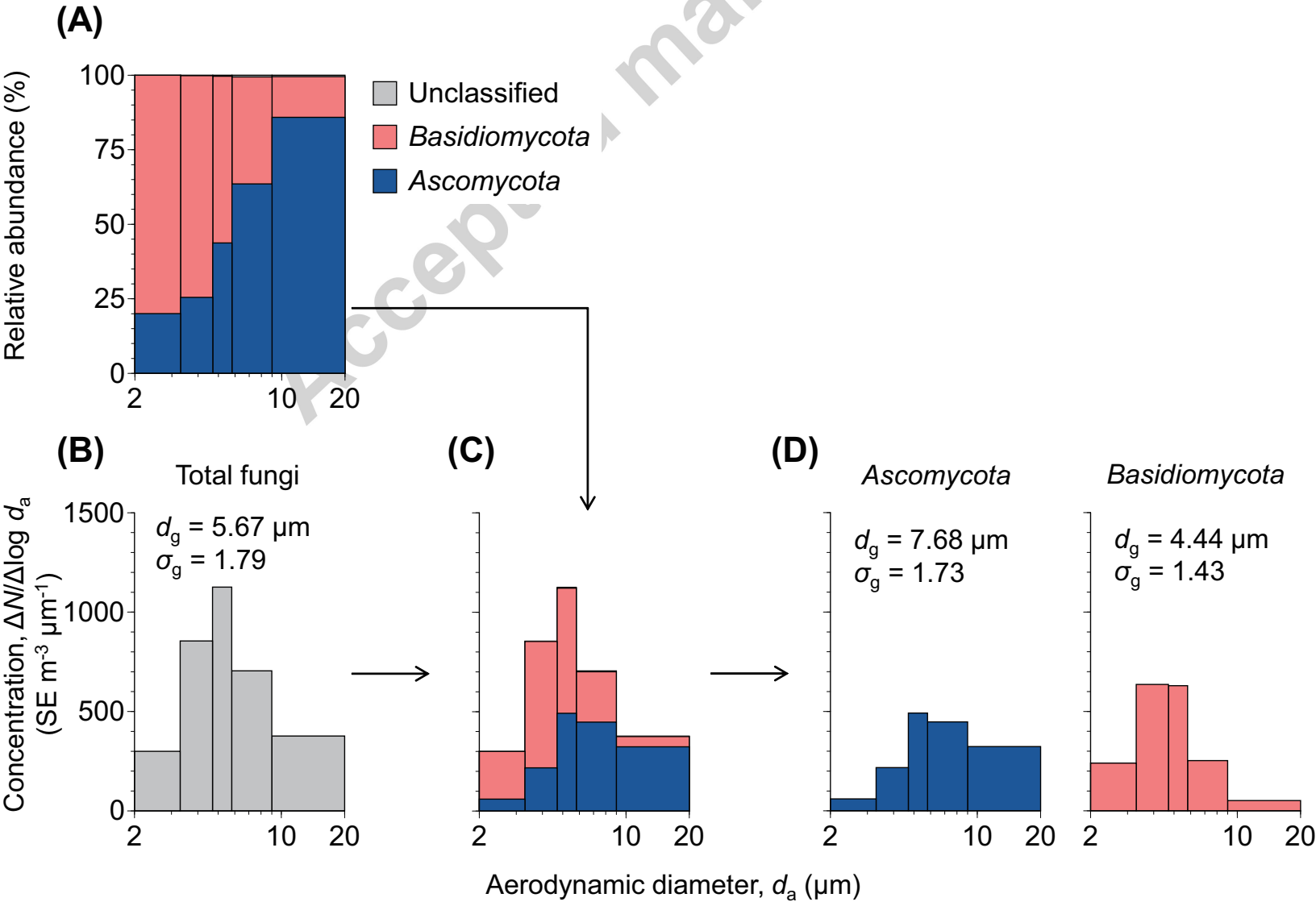

Figure 2